

HIGH SENSITIVITY MECHANICAL RESONANT SENSOR

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Technical Field of the Invention

The present invention relates generally to the field of chemical and biological
sensors and, in particular, to a high sensitivity, mechanical resonant sensor for detecting
the presence of chemical or biological material.

Background

Science and industry have developed a need for the ability to accurately detect
and measure very small quantities of chemical or biological material. Where it was
once adequate to measure quantities in micrograms, today, many applications require
the detection of a single cell, subcellular unit, or other small quantity of material,
20 sometimes on the order of 10^{-15} grams.

For example, within the food industry, even small quantities of particular
biological cells or toxins can be harmful or dangerous to mammals. One such well
recognized harmful organism is *Escherichia coli* or *E. coli* bacteria. Popular media
attention concerning the presence of *E. coli* in meat products, apple juice and alfalfa
25 sprouts has heightened consumer sensitivities.

Sensors for detecting gasses using micromechanical cantilevers are discussed in
*A Chemical Sensor Based on a Micromechanical Cantilever Array for the Identification
of Gases and Vapors*, H. P. Lang (IBM Corporation, Saumerstrasse 4, 8803 Ruschlikon,
Switzerland) et al., Applied Physics A 66, No. 5, S61-S64 (1998) (hereinafter "Lang").
30 Lang discusses detecting static beam deflection upon exposure of the beam to gases and

vapors and measuring resonance frequency shifts based on changes of beam mass due to absorption. Absorbent beams are exposed to a chemical vapor for a period of time (noted in one instance to be several hours) and measurements are taken before the chemicals have evaporated from the beam. Absorption is recognized as largely a result of Lennard-Jones potential, wherein at close distances, nearby molecules repel and at larger distances, the molecules are attracted to each other. In many cases, absorption of molecules onto a surface can be readily reversed by merely heating the system or exposing the system to a vacuum.

Biochemically induced surface stresses in a cantilever array are discussed in *Translating Biomolecular Recognition into Nanomechanics*, J. Fritz et al, Science, page 316-318, Vol. 288, April 14, 2000 (hereinafter "Fritz"). Fritz discusses absolute deflection of a beam as it relates to ligand binding in a liquid environment.

Detecting mass differences using static deflection of a beam typically requires a more robust beam. In many cases, this means that the beam is dimensionally rather large or the material of which the beam is fabricated has a relatively high Young's Modulus of elasticity. Large beams or those having high Young's Modulus of elasticity can lack the sensitivity needed to detect small quantities of target substances. In addition, beams that acquire additional mass through the process of absorption often require lengthy exposure time to the target substance to accumulate a detectable amount.

For the reasons stated above, and for other reasons stated below which will become apparent to those skilled in the art upon reading and understanding the present specification, there is a need in the art for a highly sensitive detection system and method that permits the rapid detection of biological or chemical material.

Summary

The above mentioned problems with sensors, and other problems, are addressed by the present invention and will be understood by reading and studying the following specification. A system is described for detecting an analyte. The system can include a light source, a resonating structure (such as a cantilever beam) and a photodiode. The beam has a rigid end and a free end that resonates under ambient conditions. The

resonant frequency of the beam is a function of, *inter alia*, the mass on the beam. The photodiode generates an output signal based on light reflected from the apex of the beam. The photodiode output is a function of the frequency of vibration of the beam. At least one surface of the beam includes an immobilized binding partner. The binding partner is selected so as to bind with a particular analyte, or analytes, which in turn, increases the mass of the beam.

The processor can be coupled to the photodiode and the processor can execute programming to determine the mass of analyte bound to the binding partner. The processor can include a microprocessor or a spectrum analyzer coupled to the output signal. The binding partner can be immobilized on a particular region of the beam, such as, for example near one end of the beam. The beam can be fabricated of silicon nitride. A portion of the beam can be rigidly coupled to a support. The length of the beam may be in the range of 0.5 to 1000 μm . The beam can be adjusted to vibrate in an out of plane mode. The beam can operate in an atmosphere of thermal noise or it can operate in an atmosphere of air vibrations. The beam can be tailored to detect an analyte including a pathogen, a microorganism, a bacteria, a virus or a subunit thereof. The binding partner can include an antibody that binds to a particular analyte such as a cell, a cell fragment or subunit. The binding partner can include a cellular receptor that binds to a ligand. The analyte can be a ligand specific for a cellular receptor. The analyte can be a metallic ion, or organic molecule, and the binding partner can be a chelator that binds to the metallic ion, or organic molecule. The binding partner may include a deoxyribonucleic acid ("DNA") sequence and the analyte include a complementary DNA sequence that hybridizes thereto under the operational conditions of the sensor.

A method for detecting a pathogen is also described. The method can include providing a cantilever beam, optically determining a first resonant frequency for the beam, exposing the cantilever beam to a mixture suspected of containing the pathogen, optically determining a second resonant frequency for the beam, and determining a mass difference for the beam. The beam includes an immobilized binding partner for the pathogen on a surface thereof. The first resonant frequency is determined at a time when the beam is excited by ambient conditions and the second resonant frequency is

determined at a time after the beam is exposed to the mixture. The mass difference is determined based on a difference between the first resonant frequency and the second resonant frequency.

5 The first resonant frequency can be determined by illuminating the beam using a laser light source and sensing light reflected by the beam using a photodiode. The ambient conditions can include thermal mechanical noise and ambient air vibrations. The cantilever beam can also be driven by a piezo electric oscillator. The binding partner can be immobilized by immersing the beam in a first liquid mixture of the binding partner for a pathogen, removing unbound pathogen or other components of the mixture, e.g., by rinsing with water and drying the beam in an inert atmosphere. The beam can be exposed by immersing the beam in a solution suspected of containing the pathogen, incubating the beam for a predetermined period of time, rinsing and drying the beam in an inert atmosphere. The beam can be exposed to a buffered aqueous solution. The pathogen can include a microorganism, microorganism fragment or subunit thereof. A second beam can also be prepared by immersing in a second solution suspected of containing a second pathogen. The beam can operate in a vacuum environment. The mechanical properties of the beam can be tailored to detect a mass difference in the range of attograms (10^{-18} g) to micrograms (10^{-6} g). The mechanical properties of the beam can be tailored to achieve a desired resonant frequency.

20 An array of analyte detectors is also disclosed. An array can include a plurality of cantilever beams, a plurality of immobilized binding partners and a sensor responsive to light reflected by a particular beam. Each beam resonates at a particular frequency under ambient conditions. Each beam has an immobilized binding partner on a surface. Each binding partner binds to a predetermined analyte. The sensor generates an output signal based on a resonant frequency of a particular beam.

25 At least some beams can be made of silicon nitride. A light source can be used to illuminate a beam. At least some beams in the array can have different, or heterogeneous, binding partners. At least some beams in the array can have the same, or homogeneous, binding partners.

A detector for an analyte is disclosed. The detector includes binding means, cantilever means, sensor means and processor means. The binding means are for binding with the analyte. The cantilever means are for resonating under ambient conditions. The binding means are immobilized on a portion of the cantilever means.

5 The cantilever means resonates in a first mode at a first resonant frequency. The sensor means are for determining the first resonant frequency. The processor means are for determining a mass of the analyte based on a difference between the first resonant frequency and a second resonant frequency after exposure of the binding means to the analyte.

10 The cantilever means can vibrate in an out of plane mode. The cantilever means can include silicon nitride. The sensor can include a photodiode. The processor means can include a frequency analyzer. The binding means can be immobilized at a location near an unsupported end of the cantilever means. The cantilever means can be aligned substantially horizontally. The cantilever means can be encapsulated in a vacuum.

15 The system can include a cantilever beam driver in communication with the cantilever beam. The driver can vibrate the beam at a predetermined frequency and a sensor can monitor the amplitude of vibrations of the cantilever beam. The amplitude of vibrations will vary based on the mass of the analyte on the cantilever beam. Alternatively, the driver can vibrate the cantilever beam over a range of frequencies and
20 a peak vibration amplitude of the beam corresponds to the resonant frequency of the beam.

Brief Description of the Drawings

Figure 1 illustrates one embodiment of a system according to the present subject
25 matter.

Figure 2 illustrates representative beam geometry.

Figures 3A, 3B, 3C and 3D graphically illustrate a relationship between the number of cells and a frequency differential.

Figures 4A and 4B illustrate cantilever beams with bound cells on a surface.

Figures 5A and 5B illustrate a cantilever beam having a binding partner on a surface.

Figures 6A, 6B, 6C and 6D illustrate scanning electron micrographs showing a random distribution of bound cells immobilized on the surface of four different
5 cantilever beams.

Figure 7 graphically illustrates resonant frequency as a function of detector output.

Figure 8 illustrates an array of cantilever beams.

Figure 9 illustrates a flow chart of a method pursuant to the present subject
10 matter.

Figure 10 illustrates schematically a configuration for measuring the frequency response of a beam.

Figures 11A, 11B and 11C illustrate a surface micromachining fabrication process.

Figure 12 illustrates a test setup for use with a beam fabricated using surface
15 micromachining.

Figure 13 illustrates frequency response of a beam operating in a vacuum.

Figure 14 illustrates a scanning electron micrograph of a single cell bound to an immobilized antibody layer on the surface of a beam.

Figure 15 illustrates the corresponding frequency response using the beam of
20 Figure 14.

Figures 16A, 16B, 16C and 16D illustrate alternative configurations for a resonant sensor pursuant to the present system.

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Detailed Description

The following detailed description refers to the accompanying drawings which form a part of the specification. The drawings show, and the detailed description describes, by way of illustration specific illustrative embodiments in which the invention may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the invention. Other embodiments may be used and logical, mechanical, electrical and chemical changes may be made without departing from the scope of the present invention. The following detailed description is, therefore, not to be taken in a limiting sense.

Figure 1 illustrates one embodiment of a system according to the present subject matter. In the figure, system **100** is suitable for the detection of *E. coli* or other bacterial cells. Cantilever beam **10** is affixed on one end to support **20**. The other end of beam **10** is free to move in the directions indicated by arrow **15**. Beam **10** vibrates at a resonant frequency when driven by ambient environmental conditions or when driven externally, by, for example, a piezoelectric device. In the embodiment shown, laser **30** projects light **40**, optionally through lens **35**, onto the free end of beam **10**. Lens **35** focuses the light onto the apex of beam **10**. Light **40** is reflected by beam **10**. A mirror **45** can redirect light **40** to illuminate sensor **50**. Sensor **50** generates an output signal **55** based on the vibrations of cantilever beam **10**. Spectrum analyzer **60** processes output signal **55** and yields useful information.

In one embodiment, beam **10** is fabricated of silicon nitride. Preferably, beam **10** is fabricated of low stress silicon nitride. Beam **10** may also be fabricated of other materials, including for example, silicon, silicon dioxide, silicon carbide, polysilicon, carbon, diamond like carbon (DLC) film, metal, gallium arsenide or other conductor or semiconductor material. Preferably, the material used for beam **10** is conducive to photolithography processes and etching to release beam **10** from the surrounding structure. Preferably, the material used for beam **10** is conducive to fabrication of structures having the scale and geometry as herein provided. Micromachining techniques, or other suitable technology may be used to fabricate beam **10**. Beam **10** may be fabricated using either bulk or surface silicon micromachining technology.

In the embodiment shown, beam 10 is substantially linear. Alternatively, beam 10 may include a helical section or multiple anchor points with various modes of freedom to enable greater sensitivity. Beam 10 can have different cross sectional shapes, including, for example, rectangular, square or round cross section.

5 The physical dimensions of beam 10 are selected to meet desired sensitivity requirements. Figure 2 illustrates the geometry of a typical beam 10. Preferably, beam 10 has a high aspect ratio, that is the length l , is longer than the width w , of beam 10. By way of example, but not by way of limitation, a high aspect ratio beam is one having a ratio of length to width of approximately 3.75 or more. For example, typical
10 dimensions for the length of beam 10 can be in the range of 0.5 to 1000 μm . Typical dimensions for the width of beam 10 can be in the range of 0.1 to 50 μm . A typical dimension for the thickness t , of beam 10 can be in the range of 0.05 to 4 μm . The aforementioned dimensions are not to be construed as limitations for the present system. A coordinate system is also illustrated in Figure 2, with the z-axis aligned with t , the x-
15 axis aligned with w , and the y-axis aligned with l .

In the embodiment shown in Figure 1, beam 10 vibrates in the directions of arrow 15, or substantially along the z-axis. Arrow 15 extends normal to the plane of beam 10, and thus, the vibratory mode is said to be out of plane. Other modes of vibration may also be sensed. For example, vibrations in plane may be monitored with
20 suitable sense apparatus. Vibrations in more than one plane can also be monitored.

Support 20 is coupled to one end of beam 10. In the embodiment shown in Figure 1, support 20 is illustrated as a rectangular housing. Support 20 can be a heavier region of the substrate upon which cantilever beam 10 is fabricated, and is thus stable relative to the vibrations of cantilever beam 10. Support 20 can be fabricated in
25 conjunction with the fabrication of beam 10. Consequently, support 20 may also be fabricated of the same material used in the fabrication of beam 10. In addition, support 20 may be fabricated in conjunction with other integrated electronic devices, components or circuitry. The other integrated electronic devices, components or circuitry may be related or unrelated to the operation of detector system 100. For

example, support 20 may be fabricated on the same substrate as digital logic gates, amplifiers, processors, memory cells, or other semiconductor devices.

Cantilever beam 10 vibrates at a first frequency determined by the geometry, the mass, the distribution of mass, and external forces acting on beam 10. A change in the mass of beam 10 is detectable as a change in the resonant frequency of beam 10. Figures 3A and 3B graphically illustrate this phenomena for a particular beam sensitized for detecting *E. coli* cells. The number of *E. coli* cells is shown on the abscissa and differential frequency, measured in Hertz, on the ordinate. The number of cells is proportional to the mass change of beam 10. Figure 3A corresponds to a cantilever beam 10 having dimensions of 100 micrometers (" μm ") in length, 20 μm in width and 320 nanometers (" nm ") in thickness and shows number of cells in the range of 0 to 900. Figure 3B corresponds to a cantilever beam 10 having dimensions of 200 μm in length, 10 μm in width and 600 nm in thickness and shows number of cells in the range of 0 to 160. The graphs show the substantially linear relationship between mass and frequency differential. Deviations from linearity are explained by such factors as nonuniform loading of beam 10 as well as nonuniform flexural rigidity of beam 10 resulting from variations in the distribution of the mass of beam 10. The particular beam depicted in Figures 3A and 3B was effective for detecting the presence of 16 *E. coli* cells. Figures 3C and 3D illustrate measured frequency shift dependence relative to the number of bound *E. coli* cells for particular cantilever beams. In Figure 3C, the beam has dimensions $l=15\ \mu\text{m}$ and $w=5\ \mu\text{m}$. In Figure 3D, a slightly larger beam was used having dimensions $l=25\ \mu\text{m}$ and $w=10\ \mu\text{m}$. The figures show a linear regression fit to the data.

Beam 10, and the support structure, may be fabricated using any of a number of semiconductor fabrication techniques. An exemplary bulk micromachining fabrication process is as follows:

In one embodiment, low stress silicon nitride is applied to a substrate by low pressure chemical vapor deposition ("LPCVD") to a thickness of 320 nm.

Alternatively, silicon nitride can be applied by plasma enhanced low pressure chemical vapor deposition ("PECVD") to a depth of 600 nm. Other thicknesses, as well as other

deposition technologies, are also contemplated. The substrate can be a silicon wafer. Other substrates are also contemplated, including for example but not by way of limitation, gallium (such as gallium antimonide and gallium arsenide), indium (such as indium antimonide, indium arsenide and indium phosphide), and polycrystalline materials (such as polycrystalline gallium arsenide and polycrystalline indium phosphide).

The cantilever beam **10** and support **20** are defined by photolithography on a front side of the substrate wafer. The exposed silicon nitride is etched in a reactive ion etch ("RIE") chamber using carbon tetrafluoride ("CF₄"). The back side of the wafer is treated in a similar manner with features aligned as appropriate.

To prevent roughening of the front side silicon nitride during later processing, a layer of oxide may be deposited using PECVD to a thickness of 2 μm. The wafer is etched using potassium hydroxide ("KOH"). As back side etching of the wafer approaches the front surface, the 2 μm layer of PECVD oxide is removed by buffered (6:1) oxide etch solution. The etch is continued until the cantilever is released from the surrounding structure.

In one embodiment, the release of cantilever beam **10** is aided by using a high pressure carbon dioxide (CO₂) critical point dryer. The critical point dryer reduces, or prevents, morphological damage to the structure resulting from dehydration in the atmosphere due to surface tension at the liquid interface. If left unchecked, the surface tension of the solution may aggravate stiction and result in breaking of the cantilever structure. In particular embodiments, the calculated Young's Modulus of elasticity for the silicon nitride cantilever fabricated using PECVD and LPCVD is 93 gigapascal ("GPa") and 110 Gpa, respectively.

Figures 4A and 4B illustrate one embodiment of cantilever beam **10A** having a binding partner **115** on a surface. In the figures, support **20A** is represented as a base structure and is rigidly attached to further structure not appearing in the figure. Beam **10A** has a first end **70** rigidly attached to support **20A** and a second end **80** that is cantilevered. In one embodiment, second end **80** is free to vibrate in an out of plane mode. Binding partner **115** is immobilized on beam **10A**. Binding partner **115** is

conformally distributed, or coated, on all surfaces of the structure illustrated in Figure 4A. Binding partner 115 can be localized to a particular portion of beam 10A, such as, for example, a region near second end 80. Binding partner 115 can be distributed on an upper surface of beam 10A. Binding partner 115 can be distributed on the exterior
5 surfaces of beam 10A. Binding partner 115 can be impregnated within the interior structure of beam 10A. Binding partner 115 can be a surface coating on beam 10A and thus, selectively bind to predetermined molecules. In one embodiment, binding partner 115 includes molecules 90 that bind to complementary molecules on target cells in a “lock and key” fashion. In the embodiment of Figure 4A, binding partner 115 includes
10 a plurality of antibody molecules, herein represented as a plurality of “Y” shaped characters 90. Figure 4B illustrates beam 10A having binding partner 115 at a time when complementary molecules 110 have bound with the antibody molecules 90 of binding partner 115. Binding partner 115 can bind to one or more target substances in a reversible or essentially irreversible fashion. Examples of essentially irreversible bonds
15 may include those arising by *van der Waal* forces, ionic bonds, or by formation of covalent bonds. Preferably, binding does not occur by simple physical absorption of the target by beam 10 or binding partner 115 thereon.

In Figure 4A, one embodiment of beam 10 is shown having an amount of binding partner 115 immobilized on the surface. Binding partner 115 is selected to bind
20 to a desired target substance, or substances, wherein said bound target substance, or substances, is then detected by system 100. For example, one protein (such as an antibody) may be used as a binding partner 115 on beam 10 for purposes of detecting a second protein (such as an antigen). By way of example only, and not by way of limitation, other pairs include using a receptor for detecting a ligand such as using a
25 cellular receptor to detect a ligand that binds to such receptor, using a protein for detecting a peptide, using a protein for detecting a DNA, using a first DNA sequence to detect a second DNA sequence, using a metallic ion to detect a chelator, and using an antibody, or an antibody fragment, for detecting an antigen or analyte. It will be recognized that the aforementioned examples bind to each other in a “lock and key”
30 fashion by ionic bonding, covalent bonding or a combination thereof. In some cases,

the binding partner may bind specifically to a single target substance or subunit thereof. Consequently, either the “lock” can be immobilized on beam 10 for detecting the “key” or the “key” can be immobilized on beam 10 for detecting the “lock.” As an example, a peptide may be the binding partner on beam 10 for use in detecting a protein. The
5 binding partner 115 immobilized on cantilever beam 10 can be DNA and thus, the present system is responsive to the substantial DNA complement. The bound, or “hybridized” DNA sequences can then be treated or “washed” under various conditions of stringency so that only DNA sequences that are highly complementary (e.g., that has high sequence identity) will be retained on beam 10.

10 The binding partner 115 can also bind to a plurality of substances, in which case, system 100 will indicate detection of any substance binding to cantilever beam 10. In addition, more than one binding partner 115 may be immobilized on a particular cantilever beam 10 to enable detection of multiple molecules. Multiple binding partners 115 may be immobilized in the same or different regions of cantilever beam 10.

15 The binding partner 115 can include an antibody for detection of an antigen, or binding partner 115 includes an antigen for detection of an antibody. Examples of antigens include proteins, oligopeptides, polypeptides, viruses and bacteria. For instance, antigens include OMP_a, OMP_b and OMP_c, commonly referred to as outer membrane protein “a” “b” and “c.” In such cases involving antigens, the interaction
20 includes one or more amino acid interactions wherein the amino acids are spatially arranged to form two complementary surfaces in three dimensions. Each surface includes one or more amino acid side chains or backbones.

The binding partner 115 can include an antibody for detection of a hapten, or binding partner 115 includes a hapten for detection of an antibody. Haptens tend to be
25 much smaller than antigens and include such compounds as transition metal chelators, multi-ring phenols, lipids and phospholipids. In such cases involving haptens, the interaction includes an intermolecular reaction of a surface of the hapten with one or more amino acids of the antibody, wherein the amino acids of the antibody are spatially arranged to form a complementary surface to that of the hapten.

The interaction between amino acids, such as antibody-antigen or antibody-hapten, arises by *van der Waal* forces, *Lennard-Jones* forces, electrostatic forces or hydrogen bonding. Consequently, immobilized binding partner **115** interacts with the targeted substance in a manner beyond that of simple absorption of analyte into a matrix of some type. The interaction of binding partner **115** with the target substance is characterized by rapid bonding, preferably bonding that is not reversible under ambient conditions, thus reducing the time required for reliable detection using system **100**.

Hybrid antibodies are also contemplated for either the target substance or binding partner **115**. For example, a portion of a first antibody may be cleaved and a second antibody may be bonded to the remaining portion of the first antibody, thus forming a hybridized antibody. Such an antibody may subsequently bind with two forms of antigens or haptens. As yet another example, a third antibody may be bonded to the remaining portion of the first antibody, thus enabling subsequent bonding to additional antigens or haptens. The use of hybridized antibodies in system **100** yields a detector sensitive to multiple substances and may be desirable for certain applications where detection of two or more analytes is desired.

Binding partner **115** is affixed, or immobilized, to the surface of beam **10** using any of a number of techniques, including absorption, covalent bonding with or without linker or spacer molecules or complexation. For example, in one embodiment, a cantilever beam **10** is prepared for detection of *E. coli* cells using the following method:

After releasing cantilever beam **10** from the surrounding structure, antibodies to *E. coli* were prepared and immobilized to beam **10**. The *E. coli* cells were affinity purified (by means of affinity chromatography) and isolated from a serum pool from goats immunized with whole cells of *E. coli* serotype 0157:H7 (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD). A phosphate buffer saline (0.1M NaH_2PO_4 , 0.2M Na_2HPO_4), (hereinafter "PBS"), was prepared from 1.38 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 2.84 g Na_2HPO_4 , diluted to 100ml with deionized water. *E. coli* antibody concentration of 1mg/ml was prepared by adding 1 ml of 0.3M Sodium Phosphate Buffer (pH 7.4) to 1 mg vial of antibodies. The vial was rotated until total dissolution was achieved and the solution was then incubated at 37°C for 30 minutes. The *anti-E. coli* antibodies may be

immobilized on the surface of beam 10 to an average thickness of 40 nm. Thicknesses greater than or less than 40 nm are also contemplated.

The *E. coli* O157:H7 cells were cultured in Luria broth (a nutrient broth used to support the growth of *E. coli*) and enumerated (colony forming units (“CFU”) per mL) on Luria agar. Cells were heat-inactivated by immersing 1 mL aliquots of cell culture (in 1.5 mL Eppendorf tubes) in boiling water for approximately 90 seconds. Inactivation of the cells was confirmed by spread plating 100 µL of the heat-treated cell culture onto Luria agar and plates were read after an incubation period of 24 hours at 37°C. Heat-treated cells were then pelleted by centrifugation (2040 x g for approximately 10 minutes), and re-suspended in PBS. Serial 10-fold dilutions of the re-suspended cells were performed in PBS from 10⁹ to 10⁶ CFU/mL.

After releasing beam 10 from the surrounding structure, the resonators were immersed in a solution of *E. coli* serotype O157:H7 antibodies for 5 minutes, rinsed in deionized water, and then blown dry with nitrogen. Figure 5A schematically illustrates the resulting beam 10. In Figure 5A, element 91 represents the upper surface of beam 10 and element 92 represents antibodies bound to beam 10. Following antibody coating, the cantilevers were immersed into a solution of PBS with suspended *E. coli* cells ranging in concentration from 10⁶-10⁹ *E. coli* cells/ml for approximately 15 minutes. The cantilevers were subsequently rinsed in a 0.05% solution of Tween® (ICI Americas, Inc.), rinsed in water, and then blown dry with nitrogen. Figure 5B represents the resulting beam 10. Figure 5B represents a scanning electron micrograph showing a random distribution of bound *E. coli* cells immobilized on the surfaces of several cantilevers. In Figure 5B, elements 93 and 94 represent bound *E. coli* cells.

Figures 6A, 6B, 6C and 6D illustrate scanning electron micrographs showing a random distribution of bound cells immobilized on the surface of four different cantilever beams. To reduce charging effects during scanning electron microscope (“SEM”) imaging, the samples were prepared by evaporating a thin (under 10 nm) layer of Au/Pd. Figures 6A, 6B, 6C and 6D were prepared in a similar manner, using 10⁸ *E. coli* cells per ml, and show a random distribution of the cells.

Other methods for immobilizing binding partner 115 to beam 10 are also contemplated. For example, binding partner 115 can be covalently bonded to a surface of beam 10. Binding partner 115 can also be non-covalently bonded to a surface of beam 10. Binding partner 115 can be bonded by absorption to a surface of beam 10. In particular, amino chemistry, carboxyl chemistry, and carbohydrate chemistry techniques may be used to bond binding partner 115 to a surface of beam 10.

Exposure of cantilever beam 10 to the test solution may be achieved by any suitable means. Continuing with the example of the detection of *E. coli*, one representative method includes immersing cantilever beam 10 in a buffered aqueous medium containing different concentrations of *E. coli* cells ranging from 10^6 to 10^9 *E. coli* cells/ml. The beam may be incubated in the medium at room temperature for a period of time and then rinsed, to remove unbound components of the medium, and dried in a nitrogen atmosphere. The incubation period can be approximately fifteen minutes. Rinsing may be in a solution of Tween® (ICI Americas, Inc.) to remove any loosely bound cells. Rinsing may also be in deionized water. The *E. coli* cells are not dissolved in the water.

In the embodiment shown, cantilever beam 10 resonates under ambient conditions. The geometry and dimensions of beam 10 enable measurable differences in resonant frequency without the use of external oscillatory driving forces. Ambient conditions include any thermal mechanical noise. Ambient conditions include airborne vibrations. System 100 can operate in a vacuum, thus reducing the influence of airborne vibrations.

Alternatively, however, cantilever beam 10 can resonate under external forces. External driving of cantilever beam 10 yields a greater amplitude of oscillation. In one embodiment, the driving frequency is swept through a range of frequencies and oscillation amplitude of cantilever beam 10 is measured. An example of external force includes the forces exerted by a piezoelectric driver. Magnetic drivers are also contemplated wherein a changing magnetic field drives beam 10. Other field forces may also be used to drive beam 10. For example, the support structure for cantilever beam 10 can be externally driven at a particular frequency.



With reference to Figure 1, analyzer **60** derives information from sensor **50**. Sensor **50** can include a split photodiode or two separate photodetectors and analyzer **60** can comprise a spectrum analyzer. A common spectrum analyzer presents signal amplitude information as a function of frequency. In one embodiment, the light
5 reflected from cantilever beam **10** falls first, on one portion of photodiode **50**, and second, on another portion of photodiode **50**. This alternating illumination of photodiode **50** generates an alternating output signal. Analyzer **60** receives the alternating output signal and displays information corresponding to the resonant frequency of cantilever beam **10**.

10 Figure 7 is a compilation of five representative displays appearing on the screen of analyzer **60**. The abscissa marks the resonant frequency, in kilohertz, ("kHz") and the ordinate marks the output of sensor **50**, herein labeled as Optical Detector Output and calibrated in arbitrary units. In one embodiment, the output of sensor **50** is a varying voltage, and thus, the ordinate corresponds to output voltage. The output of
15 sensor **50** can be a varying resistance, in which case, the ordinate may correspond to resistance. The lower pair of curves, marked **200** and **205** in Figure 7, corresponds to a resonating beam **10** having detected 44 cells of *E. coli* bacteria in a test solution. Curve **200** illustrates detector output as a function of frequency for beam **10** at a time after affixation of immobilized binding partner **115** as herein described and prior to exposure
20 to the test solution. Curve **205** illustrates the same beam **10** after exposure to the test solution. Resonance occurs at the frequency corresponding to the peak output. The difference between the resonant frequency of curve **200** and that of curve **205** is a function of the mass difference. In the case of curves **200** and **205**, that difference in mass represents the binding of 44 cells of *E. coli* bacteria to beam **10**.

25 Curves **210** and **215** illustrate a typical resonant frequency differential using a similar cantilever beam **10** according to the present subject matter when detecting the presence of 82 cells of *E. coli* bacteria in a test solution. Curves **220** and **225**, curves **230** and **235**, and curves **240** and **245** illustrate typical results with test media having 325, 453 and 800 cells of *E. coli* bacteria, respectively.

Figure 8 illustrates one embodiment of the present subject matter having an array of four cantilever beams, marked herein as **10B**, **10C**, **10D** and **10E**. In the figure, each of the four beams **10B**, **10C**, **10D** and **10E** are arranged in a linear manner and coupled rigidly to common support **20B**. Each beam is shown having an immobilized binding partner **115B**, **115C**, **115D** and **115E** on a surface of a beam. More or less cantilever beams assembled on a common support are also contemplated.

In the embodiment shown in the figure, each of the plurality of cantilever beams is arranged along one edge of a linear support. Other configurations are also contemplated. For example, each of the plurality of beams can be arranged on two or more edges of a geometrically shaped support or each of the plurality of beams can be arranged in a circular or oval configuration. One dimensional and two dimensional configurations for the arrangement of cantilever beams are contemplated.

Referring again to an array of cantilever beams, of which Figure 8 depicts one example, each of the binding partners **115B**, **115C**, **115D** and **115E** may be distinct from each other. In the figure, the dashed lines on the upper surface of the cantilever beams are aligned on different axis and may be interpreted as denoting different binding partners. In this manner, the cantilever beam system of Figure 8 may be used to determine if a test sample includes any element that bind with binding partners **115B**, **115C**, **115D** or **115E**.

Any suitable technique may be used to immobilize a particular binding partner **115** to a particular cantilever beam **10** within an array of cantilever beams. In one embodiment, a photoactivation technique is used to immobilize a particular binding partner **115** to a particular cantilever beam **10**. For example, light activation of a particular cantilever beam **10** can activate a photosensitive chemical coating and enable subsequent bonding of the particular binding partner **115** to the activated cantilever beam **10**. A laser light source can be used to activate a particular cantilever beam **10**. Light of a particular wavelength can also be used to activate, and affix, a binding partner **115** to a particular cantilever beam **10**. Cantilever beam **10** may be a single beam or it may be a single beam in an array of other cantilever beams **10**.

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In one embodiment, a desired binding partner **115** is applied to a particular cantilever beam **10** in an array using a manifold. The manifold can include a series of capillaries having a first end aligned to transfer a fluid to each of a plurality of cantilever beams and a second end that enables introduction of the fluid on a macro
5 level. In similar fashion, a tubule may be used to immobilize a particular binding partner on a single cantilever beam **10** or to a single cantilever beam **10** in an array of other cantilever beams **10**.

In an array configuration, at least two cantilever beams can be prepared with the same immobilized binding partner **115**. If a plurality of cantilever beams within an
10 array are prepared with each beam **10** coated with the same binding partner **115**, then the system offers broad area coverage for detecting a particular cell. In addition, using a common binding partner **115** on multiple cantilever beams **10** provides redundancy.

In an array configuration, each beam **10** in the plurality of beams in an array can have a different geometry. In particular, a first beam **10** may have a high aspect ratio
15 and a second beam **10** may have a low aspect ratio. In such a case, each beam **10** has a different resonant frequency. In an array having beams of different geometry, the binding partner **115** coating on each beam **10** may be the same or it may be different.

Detecting the frequency response of cantilever beam **10** may be achieved by any suitable means. An optical sensor can be used to detect the frequency response. One
20 embodiment of an optical sensor includes laser light reflected by a portion of the cantilever beam **10** wherein the reflected light is detected by a photodiode. The laser light may emanate from a laser diode. Preferably the light is substantially monochromatic and collimated. In an embodiment having a plurality of cantilever beams, a single laser light source illuminates multiple cantilever beams. Alternatively,
25 in an array having a plurality of cantilever beams, a single photodiode, or other sensor, monitors the frequency response of multiple cantilever beams. Each cantilever beam **10** in an array may be monitored individually by a single laser light and a single sensor. Each cantilever beam **10** in an array can be aligned to reflect light to a particular one of a plurality of photodiodes. The photodiode can include a plurality of photodiodes
30 arranged in a manner to provide output signals that corresponds to the frequency

response of a cantilever beam 10. The frequency response of a particular cantilever beam 10 within a plurality of cantilever beams may be discerned using electronic means.

Other means of deriving, or analyzing, the frequency response of a cantilever beam 10 are also contemplated. In one embodiment, movement of the cantilever beam 10 is detected based on a change in capacitance. For example, cantilever beam 10 serves as one electrode of a capacitor and a second electrode is held in a fixed position near the cantilever beam. Capacitance between the first and second electrode will vary as a function of the movement of cantilever beam 10. As another example, movement of cantilever beam 10 may be used to change the thickness, or amount, of dielectric material between beam 10 and a stationary electrode. Changes in dielectric thickness, or amount, are measurable as a frequency response. In one embodiment, piezoelectric or piezoresistive methods are used to detect the movement of cantilever beam 10. Piezoelectric detection involves generating an electric signal when the material is subjected to stress and piezoresistive detection involves sensing changes in resistance based on a stress in cantilever beam 10. Magnetic detection involves conductor movement relative to a magnetic field. Current in the conductor may be sensed. Cantilever beam 10 can serve as the moving conductor in a stationary magnetic field.

The output of the sensor can be digitized and communicated to a processor. The processor executes programming to discern the differential frequency, and thus the mass difference.

Each of the aforementioned methods of detecting the frequency response may be used in an embodiment of the present system. For example, multiple optical sensors may be used for an array of a plurality of cantilever beams. Alternatively, a single optical sensor may be used to monitor an array of a plurality of cantilever beams.

In one embodiment, an array of cantilever beams is fabricated wherein some beams are tailored to detect a first type of cell and a second set of beams are tailored to detect a second type of cell. For example, the aspect ratio of a cantilever beam may be selected to respond with greater sensitivity to a cell having a particular mass. Geometric dimensions, the method of fabrication, and the material selected for the

cantilever beam are some of the parameters that may be tailored to achieve a desired sensitivity.

In addition, the environment in which beam 10 operates has an effect on the sensitivity of the present subject matter. In the viscous regime, for example, the
5 atmospheric pressure operating on beam 10 will produce a dampening effect due to the viscosity of the air. Increased dampening effects will degrade the sensitivity of the detector. The quality factor Q of cantilever beam 10 is proportional to the inverse square root of the atmospheric pressure. In one embodiment, a beam operating in an environment of atmospheric pressure of 1 atm (approximately 760 mm Hg) and at room
10 temperature (approximately 25 °C), may have a quality factor Q of between 5 and 8. With a Q in this range, a particular beam 10 can detect approximately 44 bound cells of bacteria, such as *E. coli* bacteria. Sensitivity increases with increased quality factor Q . Increased sensitivity of the present subject matter can enable detection of both single *E. coli* bacteria and single monoatomic layers.

15 In the molecular regime, on the other hand, the quality factor is inversely proportional to the pressure. Therefore, when operated in a vacuum of 1 mTorr at room temperature, the quality factor Q is on the order of 10^4 for one embodiment. When operated in such a vacuum, the present subject matter can detect a mass in the range of 14.8×10^{-15} grams, and when operated in a standard atmosphere, can detect a mass 100
20 times larger. In addition, the mass distribution on the length of beam 10 will affect sensitivity.

The resolution of the frequency spectra is related to the width of the peak, and thus, the quality factor Q . Resolution can be 0.1Hz when operating in a vacuum and 10 Hz in standard atmosphere.

25 The sensitivity of an embodiment of the present system is a function of the slope of the relationship as illustrated in Figures 3A, 3B, 3C and 3D. In Figures 3A and 3B, the sensitivity is approximately 6.81 and 5.115 Hz/pg, respectively. Figures 3C and 3D show sensitivities of approximately 7.08 Hz/fg and 1.04 Hz/fg for a beam having $l = 15$ μm , $w = 5$ μm and $l = 25$ μm , $w = 10$ μm , respectively (l is the length and w is the width

of the beam). Sensitivities greater than 7.08 Hz/fg or less than 6.81 Hz/pg can also be achieved.

Further increases in sensitivity are also possible. For example a single *E. coli* bacteria cell as well as a single monoatomic layer can be detected, as explained herein.

- 5 A monoatomic layer of hexamethyldisiloxane (HMDS), having a mass of 14.8×10^{-15} g (that is, 14.8 femtograms), was measured using a surface micromachined cantilever beam fabricated using e-beam lithography and operated in a vacuum using an interferometric laser configuration.

Figure 9 illustrates a flow chart of a method pursuant to the present system.

- 10 Beginning at **305**, it is assumed that cantilever beam **10** has been fabricated and suitable differential frequency response detection resources are aligned. At **310**, a binding partner **115** is immobilized onto cantilever beam **10**. The immobilized binding partner **115** couples to the complementary molecule and securely holds the complementary molecule with respect to cantilever beam **10**. At **315**, a first resonant frequency is
15 determined for cantilever beam **10** along with binding partner **115**. At **320**, binding partner **115** on cantilever beam **10** is exposed to the test sample suspected of containing the complementary molecule. In one embodiment, this entails immersing the cantilever beam **10** in the test sample which can be a solution, dispersion, or suspension in an organic or inorganic liquid such as water. At **325**, a second resonant frequency is
20 determined for cantilever beam **10**, binding partner **115**, and any complementary molecules that have been immobilized by binding partner **115**. At **330**, the method continues by determining a mass difference based on the first and second resonant frequencies. Determining a mass difference may be accomplished using a look up table or by executing programming on a suitable processor. The method ends at **335**.

- 25 As previously noted, beam **10** may be fabricated by methods other than bulk micromachining. For example, beam **10** may also be fabricated using surface micromachining techniques.

- By way of comparison, bulk micromachining entails removal of a substrate by etching whereas surface micromachining entails a sequence of depositions followed by
30 selective removal of material. The material removed is defined by lithographic

techniques. Bulk micromachining techniques are complicated by backside alignment concerns as well as thickness variations across the surface of the wafer. Surface micromachining may ameliorate such issues and enable fabrication of more complex structures having smaller dimensions. Surface micromachining may also yield a more sensitive resonator.

The frequency response of a surface micromachined device may be measured using interferometric means. By way of overview, interferometric measurement entails laser light directed at surface of beam 10. A first portion of the incident light is reflected by the surface of beam 10 and a second portion of the light passes through the beam and is reflected by the underlying substrate. Vibrations of beam 10 produce an interference pattern of varying light intensity in the total reflected beam. A single photodetector cell can transduce the variations in the intensity of the reflected light. The output signal from the photodetector can be displayed on a spectrum analyzer.

One configuration for test apparatus is illustrated in Figure 10. Figure 10 also includes a magnified view of a portion of the test apparatus. In the figure, laser 30 projects incident light 40, through beam splitter 37, to beam 10. Light reflecting from the top surface of beam 10 is transmitted to beam splitter 37 and further reflected onto photodetector 50a. In addition, a portion of the incident light falling on the resonator passes through beam 10, passes through air gap 17 beneath beam 10 and is reflected by substrate 12 located under beam 10. Light reflected by substrate 12 again passes through beam 10 and is reflected by beam splitter 37 and is incident on photodetector 50a. As the beam 10 resonates, the intensity of the reflected beam is modulated by the interference of the light ray, or beam, reflecting off the surface of vibrating beam 10 and the light beam reflected off the underlying substrate. The interference patterns thus generated can be detected by photodetector 50a and an output signal from the photodetector is then applied to spectrum analyzer 60.

A profile view of the resonator is also illustrated in Figure 12. In the example shown, substrate 12 is fabricated of silicon, beam 10 is fabricated of polysilicon and between substrate 12 and beam 10 is a layer of silicon dioxide. In this case, beam 10 is semi-transparent to the incident light. In other words, a portion of incident light is

reflected by beam 10 and a portion of incident light is transmitted through beam 10. The thickness t of beam 10 is sufficiently small to render beam 10 at least partially transparent.

Optionally, a vacuum chamber, can enhance the mechanical quality factor, and thus enhance sensitivity. In Figure 10, chamber 14 provides an evacuated environment for beam 10. The vacuum environment increases the mechanical quality factor Q , thus increasing beam 10 sensitivity and ability to detect smaller masses.

The configuration shown in Figure 10 may be used to generate frequency response data using a single photocell. In contrast, the bulk micromachined configurations normally employs either two photodetectors or a conventional split cell photodiode. Using two photodetectors, or a split cell photodiode, the voltage differential between the two sensing elements provides the signal for driving the spectrum analyzer. The sensitivity of such a resonator is limited by the high speed capabilities of the instrumentation amplifiers coupled to the photodetectors, or split cell photodiode, and driving the spectrum analyzer. For example, the bandwidth of typical operational amplifiers, that is the 3 dB drop point, is in the range of several megahertz. This limitation of frequency response thus limits the sensitivity of the resonator.

Using a single photocell, on the other hand, the frequency response is not sensitive to a voltage differential, and thus, higher speed single-ended operational amplifiers can be utilized. In this case, the frequency responses may be in the gigahertz ranges. Thus, smaller, more sensitive, resonators may be possible. It has been demonstrated that using surface micromachined devices and single photodetectors, sensitivity can be in the range of attogram detection.

The surface micromachining fabrication process, illustrated in Figures 11A, 11B and 11C, begins with silicon substrate 12. With reference to Figure 11A, an 800 nm layer of silicon dioxide, layer 22, is thermally grown on silicon substrate 12 in an atmosphere of pyrogenic steam. Undoped poly-silicon, forming beam 10, is then deposited on top of the sacrificial oxide layer 22. By way of example and not by way of limitation, silicon nitride may also be used for forming beam 10. The poly-silicon is then thermally annealed at 1050° C to alleviate stresses from the residual film. Next, a

conductive 30 nm layer of chromium, layer **24**, is thermally evaporated. This
conductive layer reduces charging effects in the subsequent e-beam lithographic
definition of the resonating beam. Layer **26**, of 5.5% polymethylmethacrylate
(hereinafter "PMMA") is then spun on at 2,000 rpm for a period of 60 seconds,
5 resulting in a layer thickness of approximately 370 nm. PMMA layer **26** is soft baked
on a hotplate at 170°C for approximately 15 minutes. Figure 11A illustrates the results
of the foregoing procedure.

PMMA layer **26** is exposed using an electron beam at a dose current of 7.5 nA
and a dose of 220 $\mu\text{C}/\text{cm}^2$. The wafer is developed in 1:1 methyl isobutyl
10 ketone:isopropanol (MIBK:IPA) for 1 minute, rinsed with IPA and nitrogen (N_2) dried.
Descumming is carried out using 14 sccm O_2 at a pressure of 600 mTorr and power
level of 150 W for period of 1 minute. Figure 11B illustrates a representative profile
view.

Figure 11C illustrates the result of subsequent steps. Polysilicon is selectively
15 removed using reactive ion etching. PMMA layer **26** is stripped using oxygen plasma
and chrome (Cr) **24** is removed using a wet etch. The structure is then immersed in a
solution of hydrofluoric acid which etches the exposed silicon dioxide layer **22**, thereby
undercutting and freeing beam **10**.

Figure 12 illustrates a test setup for use with beam **10** fabricated according to the
20 above procedure. Piezo driver **32** is used to determine the resonant frequency of the
beam when operating in a vacuum bound by vacuum chamber **14**. Piezo drive **32** is
helpful with small resonators since the amplitude of vibration due to ambient noise is
very small (less than 0.01 nm). The cantilever is then coated with a self-assembled
monolayer **28** and the resonant frequency is measured. The frequency shift is then
25 correlated to the added mass.

In one embodiment, a series of beams were fabricated and a monolayer of vapor
deposited hexamethyldisilazane ("HMDS") was applied. Figure 13 illustrates the
measured frequency response. In the figure, response curve 400 represents beam **10**
before deposition of the monolayer and response curve 405 represents after

depositioning of the monolayer. The observed frequency shift was 14.5 kHz using beam **10** having dimensions of $l=4\text{ }\mu\text{m}$, $w=2\text{ }\mu\text{m}$, and $t=340\text{ nm}$. The calculated additional mass was 14.8 femtograms, and therefore, the sensitivity is approximately 1 Hz/attogram.

5 This sample was prepared in a following manner, first after the undercutting of the oxide with hydrofluoric acid ("HF"), the surface was devoid of any adsorbed water vapors. Sample was then placed inside a vacuum chamber and the first resonance peak was obtained. Sample was dehydrated at 150°C for 1 hour and immediately placed into the HMDS deposition oven. Sample was then placed into the vacuum chamber and the
10 frequency shift reported in Figure 13 was observed.

Figure 14 illustrates a scanning electron micrograph of a single cell bound to the immobilized antibody layer on the surface of beam **10**. Beam **10** had dimensions of $l=15\text{ }\mu\text{m}$, $w=5\text{ }\mu\text{m}$, and $t=320\text{ nm}$. A thin layer, under 10 nm, of Au/Pd was evaporated onto beam **10** to reduce charging effects during SEM imaging. Figure 14 illustrates the
15 corresponding frequency response measured before and after the placement of the cell on beam **10**. The frequency response measurements of Figure 15 were made under ambient conditions using the optical deflection setup. The response curves illustrate thermal and ambient noise spectra due to the transverse vibrations of beam **10**. Curve **410** illustrates the response before cell attachment and curve **415** illustrates the response
20 after cell attachment.

Various structures may be used to detect mass differences pursuant to the present system. For example, Figures 16A, 16B, 16C and 16D illustrate alternative suitable structures. In Figure 16A, the structure includes four linear members **500A**, **500B**, **500C** and **500D** extending radially from center element **510**. In one embodiment
25 as illustrated in Figure 16A, center element **510** is approximately 1 μm in length and linear members **500A**, **500B**, **500C** and **500D** are 200 nm thick and 150 nm in width. Linear members **500A**, **500B**, **500C** and **500D** are immobilized at one end by attachment to external structure. The inner ends of the four linear members support center element **510** which is free to resonate in an out of plane mode. The resonant
30 frequency of the structure can be determined by optical means operating on light

reflected, or refracted, from center element **510**. Figure 16B illustrates another embodiment also having a resonating center element and four supporting linear members. Center element **530** in Figure 16B is approximately 4 μm in length. In Figure 16B, linear elements **520A**, **520B**, **520C** and **520D** include orthogonal portions.

- 5 In Figure 16C, two arrays of linear members, or strings, are illustrated. String **550** is a representative string and is immobilized by attachment to structure **540** and **545**. In the figure, the width of the strings shown in the left array is 200 nm and those of the right array are 120 nm, and for each, the thickness is 50 nm. In the figure, the length of the strings range between 7 μm and 16 μm . In Figure 16D, a drum-shaped, or disk-shaped,
- 10 structure is illustrated. The center of drum **565** is unsupported and the perimeter of drum **565** is supported by external structure **560**. Optical detection means can be used to detect resonance of the drum structure. Alternatively, the center of the drum may be immobilized and the perimeter free to resonate. Other structures and other dimensions may be employed to yield a structure having a desired sensitivity to mass differentials at
- 15 a particular resonant frequency. Structures other than cantilever beams can be used for detecting mass differentials as herein described.

Conclusion

- Although specific embodiments have been illustrated and described herein, it
- 20 will be appreciated by those of ordinary skill in the art that any arrangement which is calculated to achieve the same purpose may be substituted for the specific embodiment shown. This application is intended to cover any adaptations or variations of the present invention.